

Extremophile Microbiology

An INL researcher samples for extremophilic bacteria and viruses in one of Yellowstone National Park's many hot springs.



Extrêmophiles are organisms that have evolved to survive conditions that were once thought to be too hostile to support any life; they literally live at life's extremes. These conditions range from scalding hot springs, to pH 1.5 acid mine drainage, to high pressure vents thousands of feet below the ocean's surface, to Antarctic ice fields, to salt flats. Biological systems are capable of performing many useful and valuable chemical reactions that are of interest to both DOE and industrially; however, the vast majority of microorganisms currently used for these applications will only operate under a very narrow range of conditions. As it is often more effective for these reactions to occur under extreme conditions, there is a great deal of interest in utilizing extremophilic microorganisms. Research at INL focuses on characterization and utilization of novel microorganisms from these environments and also examining unique products made by these organisms such as enzymes.

INL's proximity to Yellowstone National park makes it a natural sampling location that offers a range of conditions, including temperatures ranging from 50°C to over the boiling point of water, acid conditions down to pH 1, alkaline conditions up to pH 12, and high levels of many heavy metals as well as various combinations of these. Several INL researchers in Biological Sciences have sampling permits and conduct active sampling in the park. Other sites that have been sampled by INL researchers include acid mine drainage, heavy metal contaminated sites, the Nankai trough, several polar regions, Fremont glacier, and spent nuclear fuel storage pools.

INL extremophile research

Examples of research conducted by INL researchers on extremophiles include development of a thermoalkaliphilic catalase for industrial applications, development of a thermoacidophilic xylanase with bioenergy applications, and a

systems biology assessment of a thermophilic organism that produces hydrogen. These are described in more detail below. Other types of INL research involving extremophiles include studying psychro-piezo-philic (cold and pressure tolerant) organisms from the deep ocean and their role in production of gas hydrates, examining organisms found in spent nuclear fuel storage pools and their role in corrosion that occurs in those pools, understanding the role of microorganisms in causing acid mine drainage and in remediating its effects, and learning the role of microorganisms in solubilizing metals in mining operations.

Industrial applications

The thermoalkaliphilic catalase, which catalyzes the breakdown of hydrogen peroxide into oxygen and water, was isolated from an organism, *Thermus brockianus*, found in Yellowstone National Park by INL researchers. The catalase operates over a temperature

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The Energy of Innovation



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range from 30°C to over 94°C and a pH range from 6-10. This catalase is extremely stable compared to other catalases at high temperatures and pH. In a comparative study, the *T. brockianus* catalase exhibited a half life of 15 days at 80°C and pH 10 while a catalase derived from *Aspergillus niger* had a half life of 15 seconds under the same conditions. The INL catalase will have applications for removal of hydrogen peroxide in industrial processes such as pulp and paper bleaching, textile bleaching, food pasteurization, and surface decontamination of food packaging. In recognition of the potential of INL's catalase, it received an R&D 100 award in 2004.

DOE, bioenergy applications

A thermoacidophilic xylanase was isolated from another Yellowstone National Park derived organism, *Alicyclobacillus*

acidocaldarius. This enzyme catalyzes one of the major hydrolysis steps in converting hemicellulose into its component sugars. Hemicellulose comprises approximately 1/3 of biomass and represents a rich source of sugars that could subsequently be fermented to valuable fuels (ethanol) and chemicals. This enzyme represents an enabling technology that will allow biomass to be economically utilized as a viable alternative to fossil fuels to decrease U.S. dependence on foreign oil. The enzyme has activity at temperatures ranging from 30-90°C and pHs from 1-5. In recognition of the potential of the INL xylanase, it received an R&D 100 award in 2006.

Researchers at INL have been studying the fundamental biology of a thermophilic organism *Carboxydotherrmus hydrogenoformans*, isolated from thermal fields in Russia, that is able to convert carbon

monoxide in the presence of water into hydrogen and carbon dioxide. Industrially, carbon monoxide generated through steam reforming is currently converted into hydrogen and carbon dioxide catalytically by the water gas shift reaction at high temperatures (650°C). However, after the catalytic conversion process, substantial amounts of carbon monoxide remain. *C. hydrogenoformans* is able to convert even very low levels of carbon monoxide and has the potential to further increase yields of hydrogen from these processes. INL researchers have applied genomic, transcriptomic and proteomic analysis techniques to study metabolic shifts that occur under a variety of growth conditions. These studies will eventually lead to a better fundamental understanding of the biology of this organism and toward development of strategies to better control the carbon monoxide conversion process.

Selected Publications/Patents/Presentations

V.S. Thompson, K.D. Schaller, and W.A. Apel. High temperature and alkaline stable catalase. United State Patent pending.

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